ABSTRACT OF THE DISCLOSURE

The present invention relates to a novel one-well assay which couples a conventional polymerase chain reaction (PCR) amplification step to a single nucleotide primer extension step for the determination of nucleotide sequence variations in the genotyping of single nucleotide polymorphisms and other DNA variations detected by primer extension methods. A PCR amplification step, a phosphatase digestion step (or a molecular weight-selective filter step), and a primer extension step are consecutively performed in the same well plate followed by electrospray mass spectrometry detection of the single nucleotide polymorphism bases. Alternative one-well assays which utilize exonuclease I or λ-exonuclease in addition to the phosphatase digestion step are also disclosed.